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De Novo Variants in *PPFIA2* in Individuals With Neurodevelopmental Disorders

Theresa Brunet[1,2] | Michael Zech[1,2,3] | Ulrich A. Schatz[1,2] | Miriam Adamovičová[4] | Matias Wagner[1,2,3,5] | Elisabeth Graf[1,2] | Riccardo Berutti[6] | Heike Weigand[5] | Robert Jech[7] | Thomas Meitinger[1,2] | Juliane Winkelmann[1,2,3,8,9] | Melanie Brugger[1,2]

[1]Institute of Human Genetics, Klinikum Rechts der Isar, School of Medicine and Health, Technical University of Munich, Munich, Germany | [2]Bavarian Genomes Network for Rare Disorders, Munich, Germany | [3]Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany | [4]Department of Paediatric Neurology, Thomayer University Hospital, Prague, Czech Republic | [5]Department of Pediatric Neurology, Developmental Medicine and Social Pediatrics, Dr. von Hauner's Children's Hospital, University of Munich, Munich, Germany | [6]Center for Research, Development, and Advanced Studies in Sardinia (CRS4), NGS Bioinformatics Loc. Pixinamanna, Pula, Italy | [7]Department of Neurology, Charles University in Prague, 1st Faculty of Medicine, General University Hospital in Prague, Prague, Czech Republic | [8]Lehrstuhl für Neurogenetik, Technische Universität München, Munich, Germany | [9]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

Correspondence: Theresa Brunet (theresa.brunet@mri.tum.de)

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ABSTRACT

Liprin-α2, encoded by *PPFIA2*, belongs to the family of Liprin-α proteins which constitute major synaptic scaffolds participating in the assembly and maturation of synapses. Heterozygous *de novo* variants in *PPFIA2* were identified by exome or genome sequencing in two unrelated individuals with a neurodevelopmental disorder. The hypothesis of *PPFIA2* as a novel candidate gene for a neurodevelopmental disorder is supported by the gnomAD gene constraint metrics and further strengthened by our identification of seven additional individuals in large cohort studies carrying rare *de novo* variants and presenting with overlapping phenotype. In summary, we provide evidence for the second gene-disease association of a Liprin-α protein beyond *PPFIA3*.

1 | Introduction

Liprin-α proteins play an important role in the early development of the presynaptic active zone in which the assembly of large protein complexes is mediated by the recently described process of liquid–liquid phase separation (LLPS) by multiple intrinsically disordered regions (IDRs) (Kaufmann et al. 2002; Dai et al. 2006; Emperador-Melero et al. 2021; Liang et al. 2021; Xie et al. 2021). The protein family includes four members (Liprin-α1 to Liprin-α4) whose protein structures are composed of an N-terminal coiled-coil domain (CCD), central (IDRs) and three tandem C-terminal sterile alpha motif (SAM) domains (Emperador-Melero et al. 2021; Südhof 2012; Tibbe et al. 2022). Liprin-α2 and Liprin-α3 are predominantly expressed in the brain both in the pre-synaptic and post-synaptic compartments (Xie et al. 2021; Zürner and Schoch 2009; Zürner et al. 2011). Liprin-α2 encoded by *PPFIA2* has been shown to participate in presynaptic ultrastructure due to LLPS mediated by N-terminal CCD and central IDRs, and recruit components of the release machinery including CASK (Ca^{2+}/calmodulin–dependent serine protein kinase) (Spangler et al. 2013). The C-terminal SAM domains interact with kinases, phosphatases, and additional scaffolds (Xie et al. 2021; Tibbe et al. 2022) including direct interaction

with CASK via the conserved tryptophan side chain Trp981 (Pan et al. 2021).

Liprin-α3, encoded by *PPFIA3*, is the only member of the Liprin-α protein family for which a gene-disease association regarding a NDD with autism and epilepsy has recently been described (Paul et al. 2024). For *PPFIA2*, there is only a single case report describing a child with mild intellectual disability (ID) harboring a heterozygous *de novo* in-frame deletion identified by SNP array (Uehara et al. 2016). Furthermore, a review of the literature revealed an additional six individuals with rare *de novo* variants in *PPFIA2* reported in large-scale sequencing studies of patients with autism or intellectual disability (Zhou et al. 2022; Kaplanis et al. 2020; Turner et al. 2019). Here, we report two unrelated individuals with an NDD who harbor *de novo* heterozygous variants in *PPFIA2*. Our findings corroborate *PPFIA2* as a new disease gene and introduce the term α-liprinopathy for disorders caused by variants in Liprin-α proteins.

2 | Patients and Methods

Trio genome (individual 1) and proband-only exome sequencing (individual 2) were performed at the Institute of Human Genetics of the Technical University of Munich. Genome sequencing was done as part of the Bavarian Genomes project (https://www.bavarian-genomes.de/ueber-uns.html). Technical details for both individuals and a summary of rare variants identified can be found in the Supporting Information: Supplementary Methods, Figures and Tables. For individual 2, *de novo* origin of the *PPFIA2* variant was confirmed by Sanger sequencing of the parents following standard protocols.

3 | Results

3.1 | Case Descriptions

Individual 1, an 11-year-old girl from Germany, was diagnosed with nystagmus and EEG abnormalities in her first year of life. Motor milestones were achieved with delay (sitting at 13 months, walking at 3 years). Brain MRI at the age of 3 years showed coarsened gyration, immature opercularization, and a coarse corpus callosum. Furthermore, she presented with macrocephaly, muscular hypotonia, gait ataxia, ophthalmologic abnormalities, and dysmorphic facial features (Figure 1). SON-IQ (Snijders-Oomen non-verbal intelligence test) at the age of 7 years was 47, corresponding to an age of 2 years and moderate ID.

Individual 2, a 7-year-old boy from the Czech Republic, had normal motor and speech development, but was diagnosed with hyperactivity disorder. During a febrile infection at the age of 4 years, he developed severe torticollis with retroflection, dystonic-choreatic movement of the left hand and foot, and slurred speech unresponsive to levodopa. Baclofen was started with some improvement of torticollis and full restoration of vitality and speech. Currently, he still has mild cervical dystonia and some dystonic posturing. Psychological testing at the age of 4 years showed mild ID, with an actual intellectual capacity corresponding to an age of 3 years. Brain MRI, metabolic screening, blood lactate, and ophthalmologic examinations were negative. Extensive case descriptions are summarized in the Supporting Information: Supplementary Results.

3.2 | Genetic Findings

Trio genome sequencing (individual 1) detected a heterozygous *de novo* variant in *PPFIA2* as the most promising candidate. Screening of an unsolved exome/genome cohort of patients with NDDs for variants in *PPFIA2* identified a variant in another individual (Individual 2). Both variants were absent from gnomAD (v4.1.0) or gnomAD SVs (v4.1.0), respectively. In both patients, no additional single nucleotide variants or copy number variants were prioritized as potentially contributing to the observed phenotype. No additional individuals to be included were found via Genematcher at this point in time (Sobreira et al. 2015). A literature review identified seven additional patients with *de novo* variants in *PPFIA2* from large NDD cohorts, all of which are absent

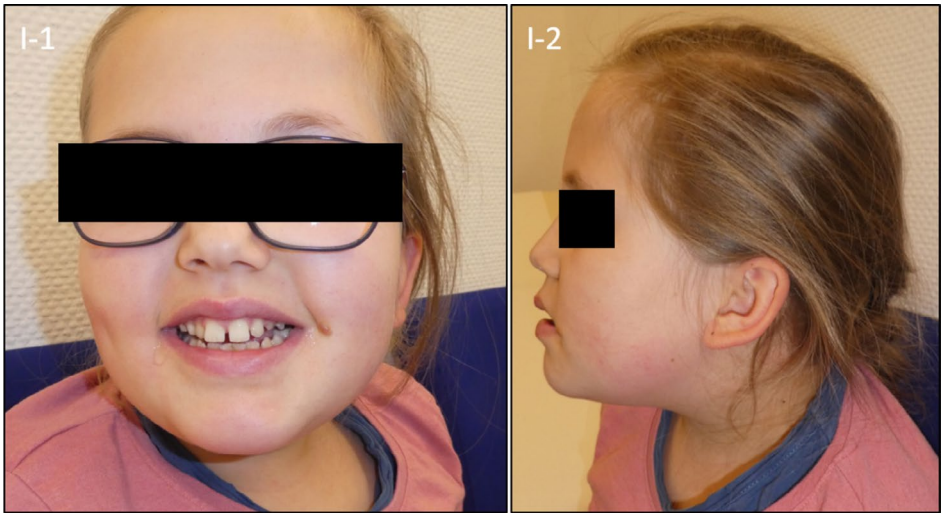

FIGURE 1 | Facial features of individual 1. Individual 1 presented with some dysmorphic features (high forehead, arched eyebrows with lateral thinning, broad nasal bridge, tent-shaped mouth, diasthema, deep set and retroverted ears).

TABLE 1 | Genetic and clinical features of individuals with heterozygous de novo variants in *PPFIA2*.

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7	Individual 8	Individual 9
Publication	This study	This study	Uehara et al. 2016	Zhou et al. 2022, Kaplanis et al. 2020	Zhou et al. 2022, Kaplanis et al. 2020	Zhou et al. 2022, Kaplanis et al. 2020	Zhou et al. 2022, Kaplanis et al. 2020	Zhou et al. 2022, Kaplanis et al. 2020	Turner et al. 2019
NM_003625.5	c.2263-797_2925+3470del	c.3367C>T	c.303+65532_645+14928del	c.2820G>C	c.1482+2T>C	c.1546G>A	c.982G>A	c.2807A>C	c.1684C>G
Exon	Exons 20–24 (in-frame)	29	Exons 5–7 (in-frame)	24	13 (splice donor site)	15	9	24	16
NP_003616.2	p.(Ile755_Thr975del)	p.(Arg1123*)	p.(Glu102_Glu215del)	p.(Gln940His)	p.?	p.(Glu516Lys)	p.(Glu328Lys)	p.(Asp936Ala)	p.(Leu562Val)
Domain	IDR, SAM 1	SAM 3	CCD1, CCD2	SAM1	CCD2	CCD2	CCD2	SAM1	—
Variant type	In-frame deletion	Nonsense variant	In-frame deletion	Missense	Splice (exon skipping would preserve reading frame)	Missense	Missense	Missense	Missense
NC_000012.11 (grch37)	g.81685145_81735785del	g.81661810G>A	Arr 12q21.31 (81818823–82005038)×1	g.81688719C>G	g.81762502A>G	g.81756563C>T	g.81777804C>T	g.81688732T>G	g.81751950G>C
Zygosity	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous
gnomAD v4.1.0	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Inheritance	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo
Method	Trio genome	Single exome and parental sanger sequencing	SNP array	Trio exome sequencing	Trio exome sequencing	Trio exome sequencing	Trio exome sequencing	Trio exome sequencing	Trio exome sequencing
ACMG classification	Variant of uncertain significance (PM2_supporting, PS2)	Variant of uncertain significance (PM2_supporting, PS2)	Variant of uncertain significance (PM2_supporting, PS2)	Variant of uncertain significance (PM2_supporting, PS2)	Variant of uncertain significance (PM2_supporting, PS2)	Variant of uncertain significance (PM2_supporting, PS2)	Variant of uncertain significance (PM2_supporting, PS2)	Variant of uncertain significance (PM2_supporting, PS2)	Variant of uncertain significance (PM2_supporting, PS2)
Age (years)	11	7	6	NA	NA	NA	NA	NA	NA
Sex	Female	Male	Male	Male	Male	Female	Male	Male	Female
Family history	Unremarkable	Unremarkable	NA	NA	NA	NA	NA	NA	NA

(Continues)

TABLE 1 | (Continued)

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7	Individual 8	Individual 9
Previous genetic testing	Chromosomes, array, *PTEN, FIX, NSD1*	Hereditary dystonia panel	NA	NA	NA	NA	NA	NA	NA
DD/ID	Moderate intellectual disability, speech delay, behavioral problems	Mild intellectual disability, hyperactivity	Mild ID	Yes	Yes	Yes	Yes	Yes	Yes
Movement disorder	Gait ataxia	Dystonia of the neck and limbs	NA	NA	NA	NA	NA	NA	NA
Facial dysmorphism	Yes	No	NA	NA	NA	NA	NA	NA	NA
Others	Macrocephaly, strabismus, nystagmus, hyperopia, pes valgus	Head circumference 50th percentile, history of uncomplicated febrile seizures	Intrauterine growth Retardation, simian crease, short fifth fingers	NA	NA	NA	NA	NA	NA
cMRI	Coarsened gyration, immature opercularization and plumped corpus callosum	Normal	NA	NA	NA	NA	NA	NA	NA

Abbreviations: DD/ID, developmental delay/intellectual disability; NA, not available.

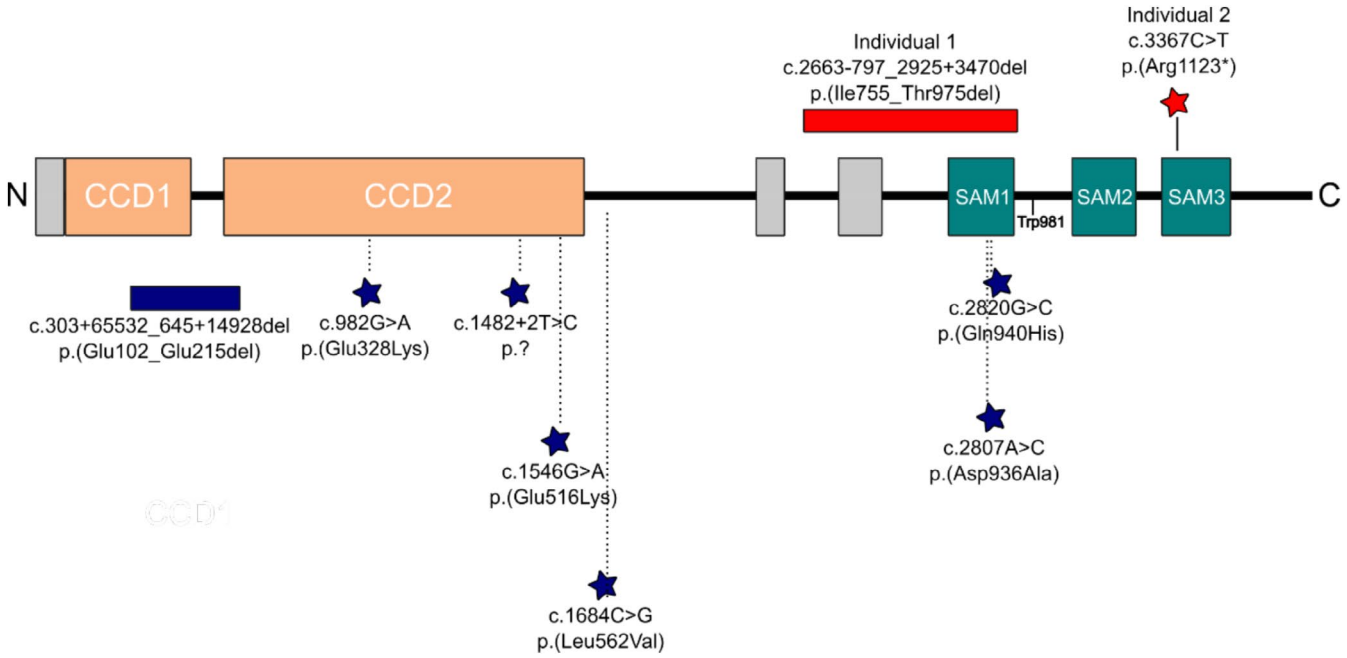

FIGURE 2 | *De novo* variants in *PPFIA2* from this study and from the literature review. Illustration of Liprin-α2 (O75334) including the two N-terminal CCDs (orange), the three C-terminal SAMs (green), the IDRs (gray) and Trp981 as binding interface for CASK (in bold) (Tibbe et al. 2022). Above, the variant localizations from the two patients from this study are shown in red. Below, the variant localizations from the patients from the literature are shown in blue.

from gnomAD (v4.1.0) (Uehara et al. 2016; Zhou et al. 2022; Kaplanis et al. 2020; Turner et al. 2019). The genotypic and phenotypic information for these individuals is summarized in Table 1 (individuals 3–9). All seven individuals presented with ID or DD. However, detailed phenotypic information was available only for individual 3, who exhibited mild intellectual disability, intrauterine growth retardation (IUGR), and a short fifth finger (Uehara et al. 2016).

Individual 1 carries a heterozygous 50.6 kb large in-frame deletion of exons 20–24 (of 33 exons; NM_003625.5) affecting the IDR and the complete SAM1. Individual 2 harbors the heterozygous nonsense variant c.3367C>T in exon 29, which is predicted to result in the premature termination of protein translation (p.Arg1123*) corresponding to the SAM3 domain. From the seven patients identified in the literature, one patient (individual 3) harbored a deletion (of exons 5–7) also predicted to preserve the reading frame and N-terminal CCDs (CCD1 and CCD2) (Figure 2). Five patients had missense variants affecting the CCD2 or SAM1 domain. One patient (individual 5) harbored a splice-site variant predicted to result in exon skipping that would preserve the reading frame. Findings of the two individuals from this study, as well as individuals from the literature, are summarized in Table 1.

4 | Discussion

PPFIA2 has been first noted as a potential disease gene in a study with 450 cases with ID. Here, we report two additional unrelated individuals with an NDD carrying heterozygous *de novo* variants in *PPFIA2*, and we extend our findings by identifying six further individuals through a comprehensive literature review.

Both reported individuals had an NDD comprising mild to moderate ID. However, other symptoms varied or were even opposite (large for gestational age reported vs. IUGR). Additionally, individual 1 showed marked dysmorphic features, whereas individual 2 showed no facial dysmorphism. Interestingly, two individuals showed signs of a movement disorder: Individual 1 had ataxia and Individual 2 had dystonia, underlining the genetic intersection between NDD and dystonia (Dzinovic et al. 2022). Movement disorder is a feature also observed in a disorder caused by variants in *CASK*, encoding CASK, which directly interacts with Liprin-α2 (Pan et al. 2021). Recently, it was shown that some missense variants in *CASK* weaken the interaction with Liprin-α2 and disrupt the regulation of Liprin-α phase separation (Tibbe et al. 2022). In turn, a potential effect of the identified variants in *PPFIA2* on the interaction of Liprin-α2 with CASK will have to be investigated.

GnomAD metrics for *PPFIA2* show a depletion of both loss-of-function variants (v4.1.0; pLI = 1; o/e = 0.27 (0.2–0.36)) and missense variants ($Z = 3.19$) (Chen et al. 2022) strongly supporting the role of *PPFIA2* as a novel disease gene. With individual 1, we describe a second patient with an intragenic deletion in addition to the patient of Uehara et al. (Uehara et al. 2016) suggesting that deletions are a common variant type for *PPFIA2*-associated disorder. Interestingly, both deletions do not disrupt the reading frame, so that a loss of certain protein components instead of nonsense-mediated mRNA decay (NMD) seems likely. The

deletion of exons 5–7 would affect parts of CCD1 and CCD2 involved in LLPS. Deletion of exons 20–24 is predicted to disrupt an IDR and the complete SAM1 which are relevant for LLPS and interacting with various kinases. Furthermore, five individuals identified in the literature harbored missense variants, indicating that missense changes are part of the *PPFIA2* variant spectrum.

Homozygous mice with *PPFIA2*-knockout developed a neurologic phenotype as well as ophthalmologic features congruent to the known functions of PPFIA2 (Guggenheim et al. 2022), but no embryonic lethality (Groza et al. 2023). Mice with a heterozygous knockout of *PPFIA2* had no apparent phenotype. As seen in a mouse model investigating possible synergistic effects of loss of multiple Liprin-α proteins (Emperador-Melero et al. 2021), double knockout mice lacking *PPFIA3* and *PPFIA2* show reduced synaptic transmission. In that work, re-introduction of *PPFIA3* in the mice could mostly rescue the recorded phenotype, suggesting a rather modulating effect of *PPFIA2*. Thus, haploinsufficiency as an underlying pathomechanism cannot yet be convincingly deduced on the basis of variant analysis alone. It will be of great interest to investigate whether the observed variants in *PPFIA2* lead to NMD or distinct impairment of PPFIA function. Besides investigating *PPFIA2* expression in brain tissue, precise characterization of functional effects of variants on PPFIA2, for example, the ability to bind interacting proteins or lead to aberrant phase separation should be explored. Studies will have to be conducted on neurons, either derived from patient-derived induced pluripotent stem cells or make use of conditional knockout models as previously performed (Emperador-Melero et al. 2021) mirroring different variant types observed in this study while accounting for possible redundancy of PPFIA1-4 function. Additionally, larger patient cohorts are necessary to better understand the disease pathomechanism, particularly variant-specific effects that may explain variable—or even opposite—phenotypes as observed in Patients 1 and 2. After *PPFIA3* has already been suggested as a new disease gene (Paul et al. 2024), we propose with the present description of *PPFIA2* the introduction of the term α-liprinopathy as a new disease entity.

Author Contributions

Conceptualization: T.B., M.Z., T.M., M.B. Data curation (clinical data): M.Z., U.A.S., M.A., H.W., R.J. Formal analysis (genetic data): T.B., M.Z., M.W., E.G., R.B., T.M., J.W., M.B. Methodology: T.B., M.Z., T.M., M.B. Supervision: T.M., J.W., M.B. Visualization: T.B., M.B. Writing – original draft: T.B., M.B. Writing – review and editing: All.

Ethics Statement

All participants or their guardians gave written informed consent for exome/genome sequencing and the publication of findings. The study was performed in agreement with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Declaration of Helsinki, and was approved by the respective local ethics committees. Additional informed consent was obtained for identifying patient images in this article.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** ajmga64255-sup-0001-Supinfo.docx.